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(57) Abstract

Method to detect specific nucleic acids of nonA-nonE hepatitis associated virus in a sample comprising the following steps: to put in contact in conditions allowing the hybridisation a GBV-C specific oligonucleotide probe either with said nucleic acids, or with amplified nucleic acids thereof, so that a nucleic acid-probe complex is formed, said probe comprising a nucleotide sequence substantially complementary to a sequence fragment of said nucleic acids, or of amplified nucleic acids thereof; to selectively detect said complex. Said nucleic acid were detected in patients suffering of nonA-nonE hepatitis but also of other pathologies, as autoimmune diseases, aplastic anaemia, haematology diseases, etc.

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METHOD FOR DETECTING NONA-NONE HEPATITIS ASSOCIATED VIRUS NUCLEOTIDE SEQUENCES, PEPTIDES AND COMPOSITIONS

The invention relates to a method to detect specific nucleotide sequences associated to nonA-nonE hepatitis virus, in biological samples, by means of sequence amplification with specific primers and detection of amplified products.

Simons et al. (Nature Medicine, 1, 564, 1995) established that a few human sera that contain antibodies recognising GBV-A and GBV-B2 recombinant proteins, harbour a third group of flavivirus-like genomes that have been named as
-GBV-C. The phylogenetic analysis of the helicase sequences of these GBV isolates indicated that these viruses can not be merely regarded as types or subtypes within the hepatitis C group but rather they constitute their own phylogenetic group.

PCT application No. WO95/21922 discloses the partial nucleotide sequence of GBV-C virus, associated to some cases of nonA-nonE hepatitis. Though a method to detect GBV virus specific nucleic acids is claimed (claim 38, p. 617), no method enabling to detect with a statistical significant percentage positive samples from nonA-nonE hepatitis subjects is disclosed. Therefore no working method for screening suspected samples or donor biological fluids is provided. PCT application No. WO95/21922 does not show nucleotide segments to be utilised for said purposes. Moreover the experiments do not demonstrate that deduced peptides can be used as probes for diagnostic assays. As a matter of fact at p. 168, table 23, peptides used in ELISA assays with nonA-nonE sera are either negative, or positive with very low percentages: 0/89 patients in Japan; 0/67 patients in Greece; 6/72 patients in USA (set M); 1/64 patients in USA (set T); 3/62 patients in USA (set 1/3); 18/32

patients in Egypt. At p. 169, table 24, only 21 out 303 nonA-nonE hepatitis patients are positive by an ELISA assay using peptides with an amino acid sequence deduced by the disclosed nucleotide sequence. It is evident that said assay can not be used neither for health patient serum screening nor for diagnosis means in suspected patients.

Yoshiba M. et al. (Lancet, 346, 1131, 1995) found what three of six cases of fulminant hepatitis of unknown aetiology were positive in a PCR assay that utilised primers derived from the NS3/helicase region of GBV-C. The Yoshiba assay requires two amplification steps (nested PCR), each one with two primers, to get an amplified product in a sufficient amount to be detected by agarose gel assay. The method is time consuming, not easy, can not be performed by non specialised personnel, and is easily contaminated. Moreover Yoshiba et al. did not clone or identify any specific probe able to selectively recognise nonA-nonE hepatitis associated sequences.

Therefore it is evident the need to provide assay methods to detect with statistical significant percentages the presence of GBV-C infection, by means of simplified procedures that are easily handled even by non specialised personnel.

The author of the instant invention has set up an assay to detect nucleic acids associated to nonA-nonE hepatitis viruses. Nucleic acids are revealed in either chronic or acute hepatitis subjects, classified as nonA-nonE for the lacking of markers associated to known viruses. Surprisingly the assay is able to detect said sequences even in patients affected by other pathologies, as autoimmune diseases, aplastic anaemia, haematology diseases, etc., thus showing a connection with GBV virus infection. The correlation index in the aplastic anaemia

affected patients show that the virus may be one of the etiologic agents.

In this context "nonA-nonE hepatitis associated virus" means nonA-nonE hepatitis associated virus having nucleotide and/or amino acid sequences able to be detected also in samples from non hepatitis affected subjects, as autoimmune diseases, aplastic anaemia, haematology diseases, etc.

The assay is preferably performed with PCR amplified nucleic acids, by means of selected primers to make only one amplification step. The amplified products are detected efficiently and reproducibly by means of selected probes able to detect very efficiently GBV-C viral sequences, even if polymorphic. The revealing method is preferably the non isotopic DEIA assay, as described in Mantero G. et al. (Clinical Chemistry 37, 422, 1991) for HBV virus sequences.

It is therefore an object of the invention a method to detect specific nucleic acids of nonA-nonE hepatitis associated virus in a sample comprising the following steps:

- to put in contact in conditions allowing the hybridisation a GBV-C specific oligonucleotide probe either with said nucleic acids, or with amplified nucleic acids thereof, so that a nucleic acid-probe complex is formed, said probe comprising a nucleotide sequence substantially complementary to a sequence fragment of said nucleic acids, or of amplified nucleic acids thereof;

- to selectively detect said complex.

According to a preferred embodiment said amplified nucleic acids are obtained by contacting in conditions allowing the hybridisation said sample with a first and a second oligonucleotide primer, both specific for nonA-nonE hepatitis associated virus, said two primers being selected from virus sequences showing no significant

sequence homology with any of HCV virus genome fragments; by making an amplification reaction. Preferably said step of contacting said sample with a first and a second oligonucleotide primer is made only once. Preferably the amplification reaction step comprises the PCR reaction (polymerase chain reaction). More preferably the PCR is performed by 45 cycles approximately, where the first is at 94°C for 5 min; at 55°C for 1 min; at 72°C for 1 min; and others are at 94°C for 1 min; at 55°C for 1 min; at 72°C for 1 min; in the presence of Mg++. More preferably PCR is made with 2mM $MgCl_2$ approximately.

According to a preferred embodiment said first primer is substantially homologous to SEQ ID No. 4 and said second primer is substantially homologous to either SEQ ID No. 5 or SEQ ID No. 6, preferably to SEQ ID No. 5.

According to a preferred embodiment the nucleotide probe comprises a sequence substantially homologous to either SEQ ID No. 7 or SEQ ID No. 8, preferably to SEQ ID No. 8.

According to a preferred embodiment the nucleotide probe is labelled and bound to a solid phase.

According to a preferred embodiment the nucleic acid-probe complex revealing step is made by means of a DEIA assay comprising the washing out of non-hybridised nucleic acids and the revealing of the nucleic acid-probe complex by means of a double helix specific ligand. Preferably said ligand is a monoclonal antibody selectively recognising double helix nucleic acids.

It is a further object of the invention a kit to detect specific nucleic acids of nonA-nonE hepatitis associated virus in a sample comprising at least:

- a first and a second oligonucleotide primer, both specific for nucleic acids of nonA-nonE hepatitis associated virus;

- a solid phase labelled nucleotide probe having a sequence substantially comprising the sequence of the amplified product by means of said first and said second;

- a double helix nucleic acid specific ligand.

According to a preferred aspect said first primer is substantially homologous to SEQ ID No. 4 and said second primer is substantially homologous either to SEQ ID No. 5 or to SEQ ID No. 6, preferably to SEQ ID No. 5.

According to a preferred aspect the solid phase labelled nucleotide probe has a sequence substantially homologous either to SEQ ID No. 7 or to SEQ ID No. 8, preferably to SEQ ID No. 8.

It is a further object of the invention a nucleic acid having a nucleotide sequence derived from the sequence of nonA-nonE hepatitis associated virus for therapy, diagnosis or immunisation, preferably of aplastic anaemia.

It is a further object of the invention a peptide having an amino acid sequence derived from the sequence of nonA-nonE hepatitis associated virus for therapy, diagnosis or immunisation, preferably of aplastic anaemia.

It is a further object of the invention a composition comprising in a pharmacologically acceptable and effective dosage form at least a peptide having an amino acid sequence derived from the sequence of nonA-nonE hepatitis associated virus for therapy, diagnosis or immunisation, preferably of aplastic anaemia.

The invention is now described for illustrating but not limiting purposes with reference to following figures, wherein:

figure 1 represents the nucleotide sequence of viral genomic portions isolated from different patients, compared to the corresponding sequence published in WO95/21922;

figure 2 represents peptide sequences as deduced by some of nucleotide sequences of fig. 1, compared to the corresponding sequence published in WO95/21922.

Inverse PCR (RT PCR)

The helicase protein of all of Flavivirus comprises at its COOH terminus the amino acid conserved sequence QRRGRTGR (SEQ ID No. 9; Muerhoff A.S. et al., J. Virol. 69, 5621, 1995). According to this sequence and to the degeneracy of genetic code an oligonucleotide primer has been synthesised:

SEQ ID No. 1: 5'GCCTGTGCKNCCNCKNCKYTG3'

where K = G or T; Y = C or T; N = A or C or G or T.

cDNA from nonA-nonE hepatitis affected patient serum RNA by means of reverse transcription was obtained. cDNA was PCR amplified (Perkin Elmer), by using as first primer an oligonucleotide of SEQ ID No. 1 and as second primer different random primers casual, unrelated to hepatitis viral sequences.

For two of cases, using primer of SEQ ID No. 1 in combination either with primer S1 of SEQ ID No. 2, or with primer bCK of SEQ ID No. 3 bands of amplification resulting to be unrelated to known hepatitis virus but related to GBV virus were obtained.

S1: SEQ ID No. 2: 5'ACGTGGATCCAAAACCTGC3';

bCK: SEQ ID No. 3: 5'AGCTTCAACAGGAATGAGTGTATGAAATACCTC3'.

According to the obtained sequences different oligonucleotides to be used as amplification primers were synthesised, and the most effective combination thereof was searched in order to detect specific nucleic acids from nonA-nonE hepatitis affected subject sera. The most effective oligonucleotides were:

H1 PRIMER (SEQ ID No.4)

5'TTATGGGCATGGHATHCCYC3'

where H = A or C or T; Y = C or T.

H2 PRIMER (SEQ ID No. 5)

5'CCRTCYTTGATGATDGARCTGTC3'

where R = A or G; Y = C or T; D = A or G or T.

H3 PRIMER (SEQ ID No.6)

5'GARCTGTCYTTVCCCCTRTAATA3'

where R = A or G; Y = C or T; V = A or C or G.

Total nucleic acids were extracted from 100 µl of serum according to standard methods and utilised either for reverse transcription or PCR, by means of random hexanucleotides (Gibco).

The antisense primer H2 and the sense primer H1 were used for the first group of 40 PCR cycles.

The antisense primer H3 and the sense primer H1 were used for the second group of 35 PCR cycles.

27 sera from acute nonA-nonE hepatitis affected subjects and 16 sera from chronic nonA-nonE hepatitis affected subjects were amplified. Amplification products having the expected length were obtained from 10 out of the acute and 6 out of chronic patients.

DEIA assay detection

Amplification products were hybridised by means of a DEIA assay with specific probes. Probe sequences were:

PR1 (SEQ ID No. 7)

5'TTCTGCCAYTCMAARGCKGAGTGYGAG3'

where Y = C or T; M = A or C; R = A or G; K = G or T.

PR3 (SEQ ID No.8)

5'GCCGGCCAGTTCTCHGCNMGGGGGGTNAATGCYATYGCCTATTA3'

where H = A or C; N = A or C or G or T; M = A or C; Y = C or T.

PR3 probe resulted to be the most effective one.

Table 1 shows the obtained results.

(Table 1 to follow)

Table 1

Clinical characterisation of GBV-C specific nucleotide sequence positive patients

Patient (age/sex)	Clinical classific.	ALT U.I.	PCR (gel)	DEIA* O.D. 450/ml
1125 (40/M)	acute	639	+	1.624
1119 (61/F)	"	1872	+	0.987
1831 (35/M)	"	710	+	1.236
1422 (36/F)	"	752	+	1.301
1159 (26/M)	"	1451	+	1.546
1124 (19/M)	"	697	+	1.123
2199 (88/F)	"	1400	+	0.789
2033 (19/M)	"	615	+	1.543
1260 (51/F)	"	2477	+	1.367
1537 (25/F)	"	725	+	1.254
1000 (45/M)	"	1435	+	1.430
2051 (33/M)	chronic	130	+	0.898
1131 (40/M)	"	127	+	1.293
2378 (60/M)	"	89	+	1.398
2938 (53/M)	"	120	+	0.835
1229 (29/M)	"	170	+	0.976
1341 (37/M)	"	93	+	0.970
3355 (46/M)	"	315	+	0.998

* threshold value = 0.350

To further confirm the high percentage of positive samples and to rule out the chance of cross contaminations among samples, amplified products were inserted in the pCRII vector (Invitrogen), and at least two independent clones were sequenced (Figure 1). Nucleotide sequences from different subjects show some differences, either among them or with sequences reported

by Simons J.N. and Yoshiba (see supra). Then data confirm an intrinsic variability of the GBV-C genome.

Though the great majority of nucleotide substitutions are silent mutations, an aligning of expected translation products revealed two main sequences groups. The former is identical to the sequence described by Simons J.N. et al. (see supra), whereas the latter, found in sera from 6 patients shows A to V and A to S substitutions, thus suggesting that at least two GBV-C subtypes exist (figure 2).

Reported data represent a minimal estimate of positive sample frequency. As a matter of fact the GBV-C RNA may be underestimated since specific PCR primers are comprised in a rather variable region of GBV-C.

GBV-C virus detection in aplastic anaemia affected patients

Total RNA was extracted from 100 µl of serum, reverse transcribed and PCR amplified, using random hexanucleotide as primers. As control RNA from health donor sera was used.

cDNA was amplified using H1 and H2 primers, with a single amplification step. The amplified product was revealed using the PR3 probe in a DEIA assay, as already described.

The GBV-C virus genome was revealed in 6 out of 10 severe aplastic anaemia affected subjects (SAA). Both of non severe aplastic anaemia affected subjects (NSAA) resulted to be negative.

Amplified products from six positive patients were cloned and sequenced. The nucleotide and the expected amino acid sequence from at least three subjects was subjected to further analysis. The total nucleotide variability is of 29.8%. The variability among subjects is in the 3.0% - 18.0% range. The expected amino acid sequence is very conserved, showing only two

substitutions (Ala→Ser at 1423 position; Glu→Gly at 1413 position) in a single clone of patient SL.

As shown in Table 2 two out six positive samples developed also hepatitis C (RS, SL) before or concomitantly with the analysis, one (MF) developed aplastic anaemia further to a cured nonA-nonE hepatitis, and three (CL, CA, B1) did not show any hepatic pathology symptoms.

Table 2

Clinical characteristics and PCR-DEIA assay results in aplastic anaemia affected patients

	patient	sex/age (years)	PCR*	DEIA**
SAA	CA	M/3	+	2.262
"	CL	M/4	+	2.271
"	FM	M/13	+	2.644
"	BI	M/16	+	2.176
"	RS	F/17	+	1.236
"	SL	M/20	+	1.855
"	LM	M/20	-	0.071
"	GS	M/47	-	0.096
"	RMP	F/53	-	0.062
"	PC	F/60	-	0.131
NSAA	ZA	F/8	-	0.050
"	BL	F/35	-	0.130

* analysed by EtBr stained agarose gel electrophoresis

** OD_{450}

All of patients were negative for HAV, Parvovirus, HCMV, Herpes Virus 1 and 2, RSV, EBV and HIV-1 markers. Three were HBV vaccinated (CL, CA, FM).

The aplastic anaemia patient positiveness is age correlated, since six out seven patients below 20 were GBV-C virus positive.

Data show a positive correlation between the severe aplastic anaemia and the GBV-C virus, not related to the hepatitis development.

The invention was described for illustrating but not limiting purposes, according to preferred embodiments, but the expert in the field may make amendments and/or variations without extending from the scope of protection of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
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(E) COUNTRY: Italy
(F) POSTAL CODE (ZIP): 20121

(ii) TITLE OF INVENTION: Method for detecting nonA-nonE hepatitis associated virus nucleotide sequences, peptides and compositions

(iii) NUMBER OF SEQUENCES: 9

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GCCTGTGCKN CCNCKNCKYT G 21

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ACGTGGATCC AAAACCTGC 19

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AGCTTCAACA GGAATGAGTG TATGAAATAC CTC 33

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TTATGGGCAT GGHATHCCYC 20

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCRTCYTTGA TGATDGARCT GTC 23

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GARCTGTCYT TVCCCCTRTA ATA 23

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TTCTGCCAYT CMAARGCKGA GTGYGAG 27

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GCCGGCCAGT TCTCHGCNMG GGGGGTNAAT GCYATYGCCT ATTA 44

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Gln Arg Arg Gly Arg Thr Gly Arg
1 5

CLAIMS

1. Method to detect specific nucleic acids of nonA-nonE hepatitis associated virus in a sample comprising the following steps:

- to put in contact in conditions allowing the hybridisation a GBV-C specific oligonucleotide probe either with said nucleic acids, or with amplified nucleic acids thereof, so that a nucleic acid-probe complex is formed, said probe comprising a nucleotide sequence substantially complementary to a sequence fragment of said nucleic acids, or of amplified nucleic acids thereof;

- to selectively detect said complex.

2. Method according to claim 1 wherein said amplified nucleic acids are obtained by contacting in conditions allowing the hybridisation said sample with a first and a second oligonucleotide primer, both specific for nonA-nonE hepatitis associated virus, said two primers being selected from virus sequences showing no significant sequence homology with any of HCV virus genome fragments; by making an amplification reaction.

3. Method according to claim 2 wherein the contacting of said sample with a first and a second oligonucleotide primer is made only once.

4. Method according to claim 3 wherein said amplification reaction step comprises the PCR reaction (polymerase chain reaction).

5. Method according to claim 4 wherein said PCR is performed by 45 cycles approximately, where the first is at 94°C for 5 min; at 55°C for 1 min; at 72°C for 1 min; and others are at 94°C for 1 min; at 55°C for 1 min; at 72°C for 1 min; in the presence of Mg++.

6. Method according to claim 5 wherein said PCR is performed with 2mM $MgCl_2$ approximately.

7. Method according to any of previous claims 2-6 wherein said first primer is substantially homologous to

SEQ ID No. 4 and said second primer is substantially homologous to either SEQ ID No. 5 or SEQ ID No. 6, preferably to SEQ ID No. 5.

8. Method according to any of previous claims wherein said nucleotide probe comprises a sequence substantially homologous to either SEQ ID No. 7 or SEQ ID No. 8, preferably to SEQ ID No. 8.

9. Method according to claim 8 wherein said nucleotide probe is labelled and bound to a solid phase.

10. Method according to claim 9 wherein said nucleic acid-probe complex revealing step is made by means of a DEIA assay comprising the washing out of non-hybridised nucleic acids and the revealing of the nucleic acid-probe complex by means of a double helix specific ligand.

11. Method according to claim 10 wherein said ligand is a monoclonal antibody selectively recognising double helix nucleic acids.

12. Kit to detect specific nucleic acids of nonA-nonE hepatitis associated virus in a sample comprising at least:

- a first and a second oligonucleotide primer, both specific for nucleic acids of nonA-nonE hepatitis associated virus;

- a solid phase labelled nucleotide probe having a sequence substantially comprising the sequence of the amplified product by means of said first and said second;

- a double helix nucleic acid specific ligand.

13. Kit according to claim 12 wherein said first primer is substantially homologous to SEQ ID No. 4 and said second primer is substantially homologous either to SEQ ID No. 5 or to SEQ ID No. 6, preferably to SEQ ID No. 5.

14. Kit according to claim 12 or 13 wherein said solid phase labelled nucleotide probe has a sequence

substantially homologous either to SEQ ID No. 7 or to SEQ ID No. 8, preferably to SEQ ID No. 8.

15. Nucleic acid having a nucleotide sequence derived from the sequence of nonA-nonE hepatitis associated virus for therapy, diagnosis or immunisation, preferably of aplastic anaemia.

16. Peptide having an amino acid sequence derived from the sequence of nonA-nonE hepatitis associated virus for therapy, diagnosis or immunisation, preferably of aplastic anaemia.

17. Composition comprising in a pharmacologically acceptable and effective dosage form at least a peptide having an amino acid sequence derived from the sequence of nonA-nonE hepatitis associated virus for therapy, diagnosis or immunisation, preferably of aplastic anaemia.

	CAT	GGT A C	ATC A T	CCC T	CTC G	GAG A	CGT A G	ATG	AGG CAA	ACT C	GGT G C A	CGC A G G A	CAC T	CTT C G	GTA G	TTC	TGC C T	CAT C	TCC A G
1229HE5	---	---	--A	---	---	---	--G	---	C--	--C	--G	A-G	---	--C	---	---	---	--C	---
1131H42IC	---	---	--T	--T	---	---	--G	---	C-A	--C	---	A-G	---	--C	---	---	---	---	---
1159HE6	---	---	--T	--T	---	---	--G	---	C-A	--C	---	A-G	---	--C	---	---	---	---	---
1160H8	---	---	--T	--T	---	---	--G	---	C-A	--C	---	A-G	---	--C	---	---	---	---	---
NNHE1	---	---	--A	---	--G	---	--A	---	C--	---	--G	A-G	--T	--C	---	---	---	--C	--A
2932H2	---	--C	--A	---	--G	---	--A	---	C--	---	--G	A-G	--T	--C	---	---	---	--C	--A
1119H1	---	--A	---	---	--G	---	--A	---	C--	---	--G	A-G	--T	--C	---	---	---	--C	--A
1124H8	---	---	--A	---	--G	---	--A	---	C--	--C	--G	A-G	---	--C	---	---	---	--C	--A
1341H19	---	---	--A	---	--G	---	--A	---	C--	--C	--G	A-G	-G-	--C	---	---	---	--C	--A
2938H3IC	---	--A	---	---	--G	---	--A	---	C--	---	--G	A-G	--T	--C	---	---	---	--C	--A
2051H1	---	--A	--A	---	--G	---	--A	---	C--	---	--G	A-G	--T	--C	---	---	---	--C	--A
2033H1	---	--A	--A	---	--G	--A	--A	---	C--	--C	---	A-G	--T	---	--G	---	---	---	--A
3355H8	---	--C	--A	---	--T	---	--G	---	C--	--T	--C	A-G	--T	--C	---	---	---	--T	--C	--A
2199H6	---	--A	---	---	---	---	--G	---	C--	--C	--G	A-G	---	--C	---	---	---	--C	---
2378H5	---	---	---	--T	---	---	--G	---	C-A	--C	---	A-G	---	--C	---	---	---	---	---
7GXBIC	---	---	---	--T	---	--A	--G	---	C--	---	--A	A-G	---	--C	--T	---	---	--C	---
G1XIC	---	--A	--A	--T	--T	---	--G	---	C-A	--C	--A	A-A	---	--C	--G	---	---	--T	--T
CXIC	---	--A	--A	---	---	---	--G	---	C-A	--C	---	A-G	---	--C	---	---	---	--T	---

FIG. 1(cont)

	AAG	GCG T	GAG	TGC T	GAG	AGA C	G	TTG C	T C	GCC T	GGC T	CAG A	TTC T	TCC T A	GCG T	C A T	CGG A	GGG A	GTT G A C	AAT C	GCC T	ATC G	T
1229HE5	--A	--T	---	---	---	C-	C	C-	T	---	---	---	---	-T-	--T		A--	--A	--C	---	--T	---	
1131H42IC	---	--T	---	--T	---	C-	G	C-	T	---	---	---	---	---	--A		A--	---	---	---	---	---	
1159HE6	---	--T	---	--T	---	C-	G	C-	T	---	---	---	---	---	--A		A--	---	---	---	---	---	
1160H8	---	--T	---	--T	---	C-	G	C-	T	---	---	---	---	---	--A		A--	---	---	---	---	---	
NNHE1	---	-T-	---	--T	---	C--		C-	C	--T	---	--A	---	---	T-	T	A--	---	---	---	---	---	
2932H2	---	-T-	---	--T	---	C--		C-	C	--T	---	--A	---	---	T-	T	A--	---	--C	---	--T	--T	
1119H1	---	-T-	---	--T	---	C--		C-	C	--T	--G	--A	---	---	T-	T	A--	---	--C	---	--T	--T	
1124H8	--A	--T	---	---	---	C-	C	C-	T	---	---	---	---	---	G-	T	A--	---	--C	---	--T	--T	
1341H19	--A	--T	---	---	---	C-	C	C-	T	---	---	---	---	---	G-	T	A--	---	--C	---	--T	---	
2938H3IC	---	-T-	---	--T	---	C--		C-	T	--T	---	--A	---	---	T-	T	A--	---	--C	---	--T	---	
2051H1	---	-T-	---	--T	---	C--		C-	T	--T	---	--A	---	---	T-	T	A--	---	--C	---	--T	--T	
2033H1	---	---	---	---	-G-	C-	C	C-	C	---	--T	---	--T	---	T-	T	A--	---	--C	---	--T	--T	
3355H8	---	-TA	---	---	--A	C-	A	---		---	---	---	---	---	---		A--	---	---	---	---	---	
2199H6	--A	--T	---	---	---	C-	C	C-	T	---	---	---	---	---	---	C	A--	---	---	--C	---	---	
2378H5	--A	--T	---	--T	---	C-	G	C-	T	---	---	---	---	---	---	T	A--	--A	--C	---	--T	---	
7GXBIC	---	---	---	---	---	C-	C	C-	A	---	---	---	--T	--A	--C	A	A-A	---	--C	---	---	---	
GXAIC	---	---	---	--T	---	C-	T	C-	C	---	---	---	--T	--T	--C		---	---	--G	---	---	--T	
CXIC	---	--T	---	--T	---	C-	G	C-	T	---	---	---	--T	---	--A	A	A--	---	---	---	---	---	

FIG. 1

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```
GBV-C      YGHGIPLERMRTGRHLVFCHSKAECERLAGQFSARGVNAIAYYRGKDS

PATIENT N°

8          ----------------------------------------------
1          ----------------------------D-----------------
1          --------------R-------------------------------
1          ------------------------G--------S------------
5          ----------------------V----------S------------
1          ---------------------VG---------S------------
1          ----------------------------C-----------------
```

FIG. 2

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IT 97/00017

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 21922 A (ABBOTT LAB ;SIMONS JOHN N (US); PILOT MATIAS TAMI J (US); DAWSON G) 17 August 1995 cited in the application Seq. Id. 709, 682 see claims ---	1-7, 9-13, 15-17
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 1997	05.06.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Molina Galan, E

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INTERNATIONAL SEARCH REPORT

Inv ional Application No
PCT/IT 97/00017

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X	JOURNAL OF MEDICAL VIROLOGY, vol. 48, no. 1, 1996, pages 60-67, XP000612659 LEARY T P ET AL: "SEQUENCE AND GENOMIC ORGANIZATION OF GBV-C: A NOVEL MEMBER OF THE FLAVIVIRIDAE ASSOCIATED WITH HUMAN NON-A-E HEPATITIS" see the whole document ---	1-6, 9-12, 15-17
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X	WO 94 18217 A (ABBOTT LAB ;TASSOPOULOS NIC C (GR); HATZAKIS ANGELOS E (GR); TROON) 18 August 1994 see the whole document ---	1-6, 9-12, 15-17
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INTERNATIONAL SEARCH REPORT

Intr onal Application No
PCT/IT 97/00017

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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